2611-Pos Board B597

Resolving Desmosomal Cadherin Interactions at the Single Molecule Level

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Desmocollin and Desmoglein are essential Ca^{2+} dependent cell adhesion proteins that mediate the integrity and functional organization of tissues in multicellular organisms; however, the molecular interactions that mediate their binding are not understood. It is currently believed that desmosomal cadherin *cis*-dimers are required to mediate adhesion, evidence for the role of *cis*-dimers is lacking. Furthermore, it is unclear if desmosomal cadherins interact via homophilic or heterophilic binding and the molecular mechanism by which desmosomal cadherins enhance their bond-strength. To resolve these questions we used Dynamic Force Spectroscopy with an Atomic Force Microscope to measure the homophilic and heterophilic binding of desmosomal cadherin monomers and *cis*-dimers at the single molecule level. Our measurements showed that desmosomal cadherin monomers alone mediate Ca^{2+} dependent adhesion, *cis*-dimers are not essential for adhesion. Furthermore, while desmocollin and desmoglein participate in Ca^{2+} dependent heterophilic binding, we could not measure Ca^{2+} dependent homophilic interactions between these cadherins. Dynamic Force Spectroscopy revealed that desmosomal cadherin interactions occur via a double barrier interaction potential; at low loading rates the outer barrier serves as the dominant impedance to unbinding while at higher loading rates the inner barrier serves as the primary kinetic barrier. These experiments resolve the molecular details of desmosomal cadherin binding and suggest a mechanism by which these essential cell adhesion molecules enhance bond strength and lifetime in the presence of force.

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Propagation of Load Through Proteins via Steered Molecular Dynamics: Effect on Fluctuation Dynamics and Architecture

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Mechanical load in the form of externally applied forces and/or moments are known to regulate biochemical activity in proteins through induced conformational changes exposing cryptic binding sites, altered kinetics, and, in extreme cases, unfolding. It is unknown, however, how load propagates through protein structure from remotely applied forces to local regions: do loads manifest as changes in fluctuations, architecture or both? Single molecule force spectroscopy (SMFS) studies have demonstrated protein unfolding with nanometer- and piconewton-level resolution, yet provide little information about the intra-protein pathways of load. SMFS studies of Green Flourescent Protein (GFP) have demonstrated the anisotropy of deformation, providing an ideal test system for studying the structural response of proteins to load. To explore the manifestation of load in proteins via simulation of a controlled experiment, constant-force steered molecular dynamics (SMD) are used to generate probability density functions (PDFs) at atomic-level resolution describing the structure of GFP when subjected to simple, sub-unfolding, externally applied loads along the vectors used in SMFS studies. Here the focus is on describing protein structure (both vibrational and architectural) with increasing forces; the experimental unfolding values provide a top-force validation of the simulation results. Total data include PDFs for both equilibrium and non-equilibrium loaded scenarios permitting comparison for the effect of loads. Data are analyzed for principal components (via PCA) to determine fluctuation dynamics and changes in structure architecture along the spectrum of applied loads. PCA data allow comparison of both fluctuation amplitude and direction through the power spectra of mode covariance overlap. Results indicate areas of increased strain under load, predicting locations of unfolding via increased strain and demonstrating the anisotropic pathways of load at sub-unfolding loads. Intra-molecular constitutive properties are calculated as derivatives of free energy.

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Naturally Occurring Osmolytes Modulate the Nano-Mechanical Properties of Polycystic Kidney Disease (PKD) Domains

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Polycystin-1 is a large transmembrane protein, which, when mutated, cause autosomal dominant polycystic kidney disease, one of the most common life-threatening genetic diseases which is a leading cause of kidney failure. PC1 is a large membrane protein that is expressed along the renal tubule and exposed to a wide range of concentrations of urea. Urea is known as a common denaturing osmolyte that affects protein function by destabilizing their structure. On the other hand, it is known that the native conformation of proteins can be stabilized by protecting osmolytes which are found in the mammalian kidney. PC1 has an unusually long ectodomain with a multimodular structure including 16 Ig-like polycystic kidney disease (PKD) domains. Here we used single-molecule force spectroscopy to directly study the effects of several naturally occurring osmolytes on the mechanical properties of PKD domains. This experimental approach more closely mimics the conditions found *in vivo*. We show that upon increasing the concentration of urea there is a remarkable decrease in the mechanical stability of human PKD domains. We found that protecting osmolytes such as sorbitol and TMAO can counteract the denaturing effect of urea. Moreover, we found that the refolding rate of a structurally homologous archaeal PKD domain is significantly slowed down in urea, and this effect was counteracted by sorbitol. Our results demonstrate that naturally occurring osmolytes can have profound effects on the mechanical unfolding and refolding pathways of PKD domains. Based on these findings, we hypothesize that osmolytes such as urea or sorbitol may modulate PC1 mechanical properties and may lead to changes in the activation of the associated Polycystin-2 channel or other intracellular events mediated by PC1.

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2614-Pos Board B600

Titin Domains as Mechanical Reporters of UNC-45 Mediated Myosin Folding

Andres Oberhauser, Christian Kaiser, Liang Ma, Henry Epstein.

Myosins are protein machines in which allosteric cross-talk between ATP-binding and hydrolysis and the movement of actin filaments requires precise changes in the polypeptide fold of the motor domain. UNC-45, a member of the UCS family of proteins, acts as a chaperone for myosin and is essential for proper folding and assembly for myosin into muscle thick filaments *in vivo*. However the molecular mechanisms by which UNC-45 interacts with myosin to promote proper folding of the myosin head domain are not known. We have devised a novel approach to analyze the mechanical refolding of the myosin motor domain at the single molecule level utilizing AFM techniques. By chemically coupling an I27 titin polyprotein to the motor domain of myosin, we introduced a ''molecular reporter'' into the motor domain, providing a specific attachment point and a well characterized mechanical fingerprint in the AFM measurements. This approach enabled us to study the folding of the motor domain and directly observe the effect of the chaperone UNC-45. The motor domain misfolded after mechanical unfolding, whereas the myosin rod refolded independently of the motor domain. The misfolded motor domain recruited the otherwise robustly folding I27 modules into a misfolded state. Thus, the I27 domains function as a folding sensor. UNC-45 prevents the detrimental interaction between the motor domain and the I27 modules, indicating that it bound to and stabilized the non-native myosin motor domain. Using truncated versions of UNC-45, we localized this protective effect to the C-terminal UCS domain of UNC-45. Our approach enables the study, for the first time, the chaperone effects of UNC-45 on myosin folding in mechanistic detail.

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Tensile Mechanical Measurements on Individual Collagen Fibrils

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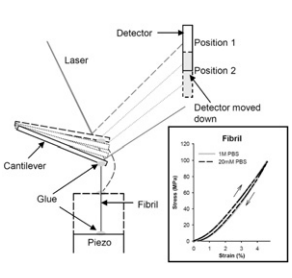
Introduction:

There is a hierarchical order in tendon ranging from the collagen molecule to the whole tendon. We have developed a method using the atomic force microscope (AFM) together with a novel data acquisition technique to quantify the sub-failure mechanical properties of individual collagen fibrils. Here we use this method to investigate how the saline environment affects tensile force transmission.

Methods:

Human patellar tendon tissue was obtained from a healthy young male. Six fascicles and six fibrils were mechanically tested sub-failure to determine modulus and relative energy dissipation in PBS solutions of 0.02, 0.15 and 1 M concentration as well as two HEPES buffers containing NaCl or NaCl+$CaCl_2$. Mechanical testing was performed to 4% strain.



Results:

The tensile properties of collagen fibrils are highly resistant to changes in their saline environment. The only consistent response to environment composition was a minor (<5%) increase in relative energy dissipation in 1 M PBS. This is in sharp contrast to previous reports of more than 100% increase in radial compressive modulus of fibrils in 1 M salt solution, and more than 100% increase in energy dissipation of AFM pulling experiments in the presence of Ca^{2+}.